

Analysis of Lignin-Polysaccharide Complexes Formed during Grass Lignin Degradation by Cultures of *Pleurotus* Species

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A brown material, precipitable with ethanol, was formed during wheat straw and lignin degradation by liquid cultures of different species of *Pleurotus*. Fourier transform infrared spectroscopy and cross-polarization and magic-angle-spinning ¹³C nuclear magnetic resonance spectroscopy showed that most of the precipitable material was formed from exopolysaccharide secreted by the fungus but it also contained an aromatic fraction. The results of acid hydrolysis, methylation analysis, and Smith degradation indicated that the major exopolysaccharide produced by these fungi is a (1→3)-β-glucan branched at C-6 every two or three residues along the main chain. The presence of lignin or straw in the culture medium had little effect on the composition and structure of the extracellular polysaccharide. Cross-polarization and magic-angle-spinning ¹³C nuclear magnetic resonance spectroscopy provided an estimation of the aromatic content of the lignin-polysaccharide complexes, assigning 20% of the total ¹³C signal in the material recovered from cultures of *Pleurotus eryngii* in lignin medium to aromatic carbon. Analytical pyrolysis indicated that the aromatic fractions of the lignin-polysaccharide complexes were derived from lignin, since products characteristic of pyrolytic breakdown of H (*p*-hydroxyphenylpropane), G (guaiacylpropane), and S (syringylpropane) lignin units were identified. These complexes cannot be fractionated by treatment with polyvinylpyrrolidone or extraction with lignin solvents, suggesting that the two polymers were chemically linked. Moreover, differences in composition with respect to the original lignin indicated that this macromolecule was modified by the fungi during the process of formation of the lignin-polysaccharide complexes.

In spite of recent progress in the study of the enzymes responsible for lignin degradation (14, 33), effective depolymerization of this macromolecule by fungal enzymes has not been achieved in vitro. To understand lignin biodegradation, it is important to bear in mind that to our knowledge, the fungal ligninolytic system bears great similarities with lignification in vascular plants in terms of the enzymes involved and the type of reactions produced (13). Peroxidases seem to be responsible for the key steps triggering both lignin biosynthesis and biodegradation processes. As a result of the action of these peroxidases, aromatic free radicals are formed, which undergo condensation reactions in biosynthesis and lead to the cleavage of intermonomer linkages in biodegradation. Consequently, ligninolytic fungi must have mechanisms for changing the spontaneous tendency to polymerize into degradation.

For *Phanerochaete chrysosporium*, the model organism for lignin biodegradation studies, the synthesis of a hyphal sheath of polysaccharide during lignin degradation has been described (20). Lignin added to liquid cultures becomes bound to this hyphal sheath (21). The immobilization of lignin close to the fungal hypha could facilitate depolymerization by oxidative reactions catalyzed by ligninolytic peroxidases, which have been reported to be immobilized in the hyphal sheath (30). Simultaneously, the degradation products would tend to be incorporated into the polysaccharide sheath (17), limiting the repolymerization tendency. Finally, the degradation of the lignin-polysaccharide complexes formed would proceed through the combined action of hydrolytic and oxidative reactions (15), concluding with complete mineralization.

In the present study, the formation of extracellular lignin-polysaccharide complexes during wheat lignin degradation by fungi from the genus *Pleurotus* and the nature of these complexes are described. Among these fungi, *Pleurotus eryngii* is being actively investigated because of its ability to degrade grass lignin selectively (i.e., causing limited cellulose degradation) (24).

MATERIALS AND METHODS

Production of lignin-polysaccharide complexes. The six species of *Pleurotus* studied, *P. cornucopiae*, *P. eryngii*, *P. floridanus*, *P. ostreatus*, *P. pulmonarius*, and *P. sajor-caju*, were cultivated in N-limited glucose-ammonium medium supplemented with 0.1% wheat alkali-lignin (23) or 1% milled wheat straw. The origin of the fungal strains, the media, and the inoculation and culture conditions have been described elsewhere (10). The polysaccharide concentration was measured with the phenol-H₂SO₄ reagent (5). Lignin degradation in the alkali-lignin medium was estimated by measuring the decrease of A₂₈₀.

After 20 days of culture, the mycelia were removed by centrifugation and ethanol was added to a final concentration of 40%. The precipitate was recovered, dialyzed against water, dissolved, and reprecipitated with ethanol. Part of this material was freeze-dried for acid hydrolysis, Fourier transform infrared spectroscopy (FTIR), cross-polarization and magic-angle-spinning ¹³C-nuclear magnetic resonance (CPMAS ¹³C-NMR), and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The rest was maintained in ethanol until used for methylation analysis. The identification of low-molecular-weight products in the culture medium has been reported previously (10).

Polyvinylpyrrolidone fractionation. Commercial insoluble polyvinylpyrrolidone (PVP; Polyclar AT) (Serva) was used in fractionation experiments. The ethanol precipitate was dissolved in 0.1 M NaOH, neutralized with 0.1 M HCl, and added to PVP (which had been successively washed with 0.1 M NaOH and 0.1 M HCl). After 5 min of contact time, PVP was separated by centrifugation and a nonretained fraction was obtained. The PVP was washed with 0.1 M HCl and treated with 0.1 M NaOH, which released the PVP-retained fraction. Both fractions were dialyzed, dried (45°C under vacuum), and analyzed by FTIR, as described below. Laminarin (Sigma), fungal polysaccharide (from glucose medium), and wheat alkali-lignin were treated with PVP in the same way.

Acid hydrolysis, methylation analysis, and Smith degradation. Freeze-dried polysaccharides were hydrolyzed with 5 M trifluoroacetic acid for 16 h at 100°C, and the monosaccharides obtained were identified as alditol acetates by GC with

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TABLE 1. Lignin degradation and production of precipitable extracellular material by six *Pleurotus* species

<i>Pleurotus</i> sp.	Lignin degradation (%) ^a	Amt (mg/liter) of extracellular material ^b in:		
		GL	LI	ST
<i>P. cornucopiae</i>	89	202	84	161
<i>P. eryngii</i>	77	49	134	198
<i>P. floridanus</i>	88	199	145	238
<i>P. ostreatus</i>	91		174	
<i>P. pulmonarius</i>	79	277	410	654
<i>P. sajor-caju</i>	85	285	143	238

^a Results from measurement of A_{280} in the lignin medium.^b Estimated after ethanol precipitation from glucose (GL), lignin (LI), and straw (ST) media and freeze-drying.

a flame ionization detector (Perkin-Elmer Sigma 3) (19). The absolute configuration was determined by the method of Gerwig et al. (9).

Because of the insolubility of freeze-dried polysaccharides, the methylation analysis was performed with polysaccharide precipitated in ethanol and dialyzed against water. The water solution was concentrated by covering the dialysis bag with polyethylene glycol 20000 (Merck). Dimethyl sulfoxide was added to the concentrated polysaccharide, and the solution obtained after shaking 8 h at 60°C was concentrated up to 5 mg/ml (under vacuum). After water traces were removed by dialyzing the concentrated polysaccharide solution against dimethyl sulfoxide with a molecular sieve (pore size, 0.4 nm; Merck), the polysaccharide was methylated with methyl iodide (27), hydrolyzed with 5 M trifluoroacetic acid (for 8 h at 100°C), reduced with sodium borodeuteride, and acetylated. The partially methylated alditol acetates were analyzed by GC-MS (Perkin-Elmer) with an SP-2340 column (30 m by 0.25 mm; film thickness, 0.2 μ m), a temperature program (10°C/min) from 200°C (3 min) to 230°C (15 min), and an ion trap detector.

Polysaccharide samples were oxidized with 15 mM sodium metaperiodate, reduced with sodium borohydride, and subjected to mild hydrolysis at 50°C (1 M H_2SO_4 for 24 h) to remove residues of oxidized sugars (Smith degradation). The modified polysaccharide was methylated and analyzed as described above.

Inositol (1 mg/ml) was used as the GC internal standard after acid hydrolysis of both native and methylated polysaccharides.

FTIR and CPMAS ^{13}C -NMR analyses. FTIR spectra were obtained with a Bruker IFS 28 spectrometer, using 1 mg of freeze-dried samples and 300 mg of KBr (100 scans were accumulated with 2-cm⁻¹ resolution).

Solid-state ^{13}C -NMR spectra were obtained with a Bruker MSL-300 spectrometer operating at 75.4 MHz by using the CPMAS technique under quantitative conditions (8). The pulse repetition rate was 5 s, the cross-polarization contact time was 1 ms, the sweep width was 31.25 kHz, the filter width was set to 37.5 kHz, and the acquisition time was 0.016 s. Magic angle spinning was performed at 4 kHz in double-bearing probes in phase-stabilized ZrO_2 rotors. The chemical shifts were calibrated with glycine.

Py-GC-MS. Samples (0.1 to 1 mg) were pyrolyzed in a quartz holder with a heated-filament pyrolyzer (Pyroprobe 1000) at 600°C for 5 s. The pyrolyzer was connected to a gas chromatograph (Varian 3400), which in turn was coupled to an ion trap detector (Magnum; Finnigan). The GC column was a Supelco SPB-5 column (30 m by 0.32 mm) operating from 50 to 290°C at 5°C/min, with the initial temperature held for 10 min. The injector was at 250°C in the split mode (1/120 split ratio). Mass spectra were recorded under electron impact at 70 eV from 40 to 400 m/z (1 scan per s). Peak identification was based on the National Bureau of Standards library, on mass-spectral interpretation, and on previous Py-GC-MS analyses of lignocellulosic materials (28, 29).

RESULTS

Production of polysaccharide-type material. The production of extracellular polysaccharide-type material was observed in liquid cultures of the six species of *Pleurotus* studied (Table 1). Moreover, the six fungi strongly degraded lignin added to the culture medium. In the straw medium, intense fungal growth was observed; however, since the straw acted as a support for fungal pellets, it was impossible to estimate degradation. The highest production of polysaccharide-type material (nearly 700 mg/liter by *P. pulmonarius*) was also obtained after addition of straw.

The polysaccharide-type material that precipitated from the straw medium and especially from the lignin medium was

brown. The fraction responsible for the color seemed to be bound to the polysaccharide, since it was recovered after dissolution and reprecipitation with ethanol and was not extracted with dimethyl formamide (a reasonable lignin solvent). To check coprecipitation, successive ethanol and acid precipitations were carried out in a solution containing straw alkali-lignin and fungal polysaccharide (from glucose medium) in concentrations similar to those found in culture filtrates. The FTIR spectrum of the ethanol precipitate revealed the presence of only polysaccharide, whereas lignin was found after acid pH precipitation. Moreover, the brown material recovered from lignin and straw media was treated with PVP, a product known for its ability to bind phenolic compounds (22). FTIR spectra showed that the brown material retained by PVP had a similar composition to the original lignin-polysaccharide complex. Since previous experiments demonstrated that PVP retained lignin but did not bind fungal polysaccharide or laminarin, the results obtained also supported the existence of chemical linkages between the fungal polysaccharide and the brown fraction. Since analyses discussed below suggest that the latter material is derived from lignin, the name "lignin-polysaccharide complexes" was used for the entire precipitable material obtained from the cultures of *Pleurotus* species in straw- and lignin-containing media. The analysis of its composition was one of the objectives of this study, and, for this purpose, hydrolysis, methylation analysis, spectroscopic methods, and Py-GC-MS were used.

Acid hydrolysis, methylation analysis, and Smith degradation. The polysaccharides produced by the different species of *Pleurotus* consisted mainly of D-glucose (95%). The small amounts of D-mannose and D-galactose corresponded to a minor fraction, which was removed from the main glucan by taking advantage of its higher solubility in water. The methylation analysis showed only minor differences between polysaccharides produced in the different media, as shown for *P. eryngii* (Table 2). The polysaccharide fraction consisted of a glucan formed by (1→3)-linked glucopyranosyl residues, 25 to 33% of them branched at C-6. A similar percentage of terminal units was detected. Moreover, a nearly linear glucan (as evidenced by methylation analysis) was obtained after one cycle of Smith degradation (which removed terminal units), indicating that over 90% of glucan branches consisted of a single glucose residue.

IR spectroscopy. The FTIR spectra of the lignin-polysaccharide complexes produced by *P. eryngii* in glucose, lignin, and straw media are shown in Fig. 1. For comparative purposes and to facilitate the identification of lignin bands in these materials, the spectrum of wheat alkali-lignin is also presented.

The band patterns shown in Fig. 1A and C confirmed the composition and types of linkage in the polysaccharide fraction: the 890-cm⁻¹ band indicated the β configuration of the main linkages, and those at 1,640, 1,420, 1,370, 1,315, 1,250, 1,200, 1,150, 1,075 and 1,040 cm⁻¹ are typical of (1→3)-glucans (26). The amide band at 1,535 cm⁻¹ (and the intensity of the

TABLE 2. Methylation analysis of the polysaccharide fraction produced by *P. eryngii*

Type of unit	Molar abundance (%) in ^a :		
	GL	LI	ST
Glc-(1→	29	34	33
→3)-Glc-(1→	44	37	39
→3,6)-Glc-(1→	27	29	27

^a Glucose (GL), lignin (LI), and straw (ST) media.

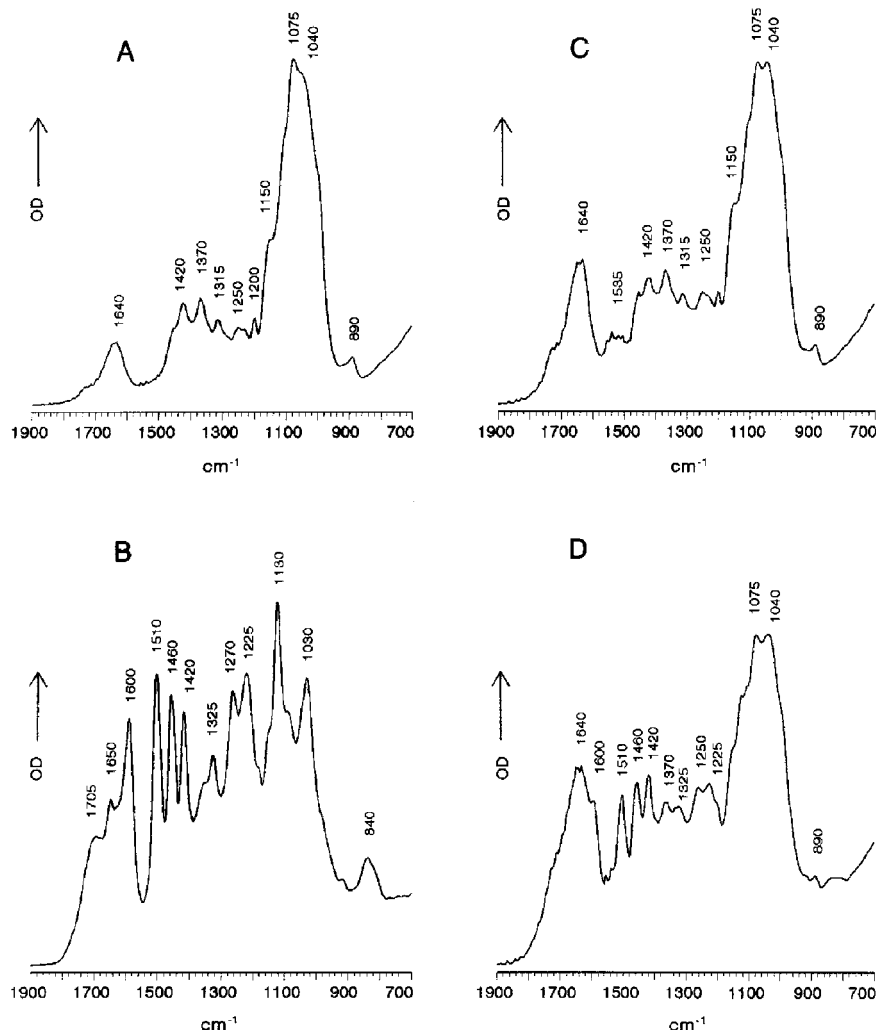


FIG. 1. FTIR spectra (1,900- to 700-cm⁻¹ region) of the extracellular material isolated from cultures of *P. eryngii* in glucose (A), straw (C), and lignin (D) media and wheat lignin (B). See the text for a description of band assignments.

band at 1,640 cm⁻¹) in the spectrum of the complex obtained from the straw medium (Fig. 1C) suggests the presence of some protein (hardly detected in Fig. 1A and D).

The spectrum in Fig. 1B shows bands characteristic of grass lignin (including ether-linked ferulic acid) (6, 12), consisting of *p*-hydroxyphenylpropane (H), guaiacylpropane (G), and syringylpropane (S) units: 1,600, 1,510, 1,460, and 1,420 cm⁻¹ (aromatic ring), 1,650 and 1,705 cm⁻¹ (conjugated and non-conjugated carbonyl groups), 1,325 and 1,270 cm⁻¹ (S and G rings), 1,225 cm⁻¹ (phenolic OH), 1,130 cm⁻¹ (S ring), and 1,030 cm⁻¹ (methoxy groups and G ring). Bands at 1,510 and 1,460 cm⁻¹ were useful in demonstrating the presence of lignin in complex substrates, since they do not overlap with bands from other natural polymers. These two bands are evident in the spectrum in Fig. 1D, suggesting that the material recovered from the lignin medium has a significant aromatic content. However, only a small peak at 1,460 cm⁻¹ can be observed in the spectrum in Fig. 1C (straw medium).

Solid-state NMR. CPMA¹³C-NMR spectra of the lignin-polysaccharide complexes produced by *P. eryngii* in glucose, lignin, and straw media are compared in Fig. 2. As in Fig. 1, the wheat lignin spectrum is also presented. The signals in the

spectra in Fig. 2A and C correspond to different carbons in (1→6)-branched (1→3)-β-glucans: 104 ppm (C-1), 86 ppm (substituted C-3), shoulder at 76 ppm (C-5), 74 ppm (free C-3), 68 ppm (free C-4), and 63 ppm (free C-6). The position of the anomeric carbon signal around 104 ppm (103.1 ppm in ¹³C-NMR spectra of glucan dissolved in deuterated dimethyl sulfoxide [data not shown]) confirmed the β configuration of the glucan. The free C-2 and substituted C-6 signals would overlap, because of wide bands obtained in the solid state (31, 32). The carbonyl signal (172 ppm) in the spectrum in Fig. 2C could correspond to oxidized lignin units (but it could be due also to the presence of some protein or ether-linked ferulic acid). The small signal at 33 ppm could also correspond to protein.

The NMR spectrum of lignin showed the characteristic signals of the carbons in the different structures of this macromolecule: 162 ppm (C-4 in H units), 152 ppm (C-3 and C-5 in etherified S), 147 ppm (C-3 and C-4 in etherified G, and C-3 and C-5 in phenolic S), 133 ppm (C-1 and C-4 in S, and C-1 in G), 74 ppm (C_α in units with β-O-4 linkages), and 55 ppm (methoxy groups) (25). The same lignin signals are evident in the spectrum of the lignin-polysaccharide complex recovered from lignin medium (Fig. 2D) but they are practically absent

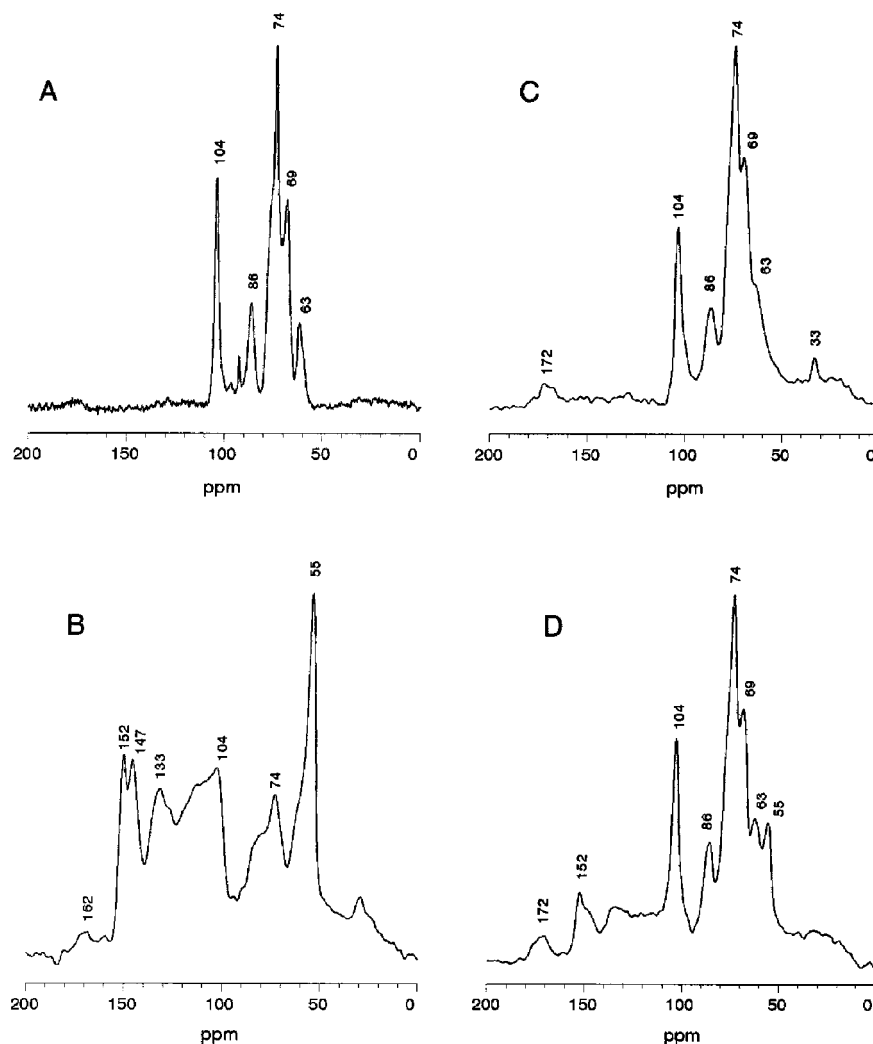


FIG. 2. CPMAS ^{13}C -NMR spectra of the extracellular material isolated from cultures of *P. eryngii* in glucose (A), straw (C), and lignin (D) media and wheat lignin (B). See the text for a description of band assignments.

from the spectrum of Fig. 2C, corresponding to the material recovered from the straw medium (as well as from the spectrum of glucan synthesized in glucose medium [Fig. 2A]).

The signals corresponding to different carbons in (1 \rightarrow 6)-branched (1 \rightarrow 3)- β -glucan, to aromatic-C, and to methoxy groups were integrated. From the intensities of the signals of substituted (86 ppm) and unsubstituted (74 ppm) C-3, the existence of one branch every two units of the glucan main chain was deduced. This agreed with information obtained from the methylation analysis, showing one branch every two or three units of the main chain. The aromatic-C content of the lignin-polysaccharide complex obtained from the lignin medium, calculated from the integration of the 160- to 110-ppm region in Fig. 2D, approached 20% (and the content of lignin-type methoxyphenylpropane structures could be estimated at 30%).

Analytical pyrolysis. Some products from carbohydrate pyrolysis, including hydroxymethylfurfaldehyde (peak 10), anhydroglucopyranose (peak 19), and several minor peaks (peaks 1, 2, 4, 5 and 7), can be recognized in Fig. 3A, but they have limited diagnostic value. Peaks 10 and 19 were also the major

products of pyrolysis of the lignin-polysaccharide complex from the straw medium (Fig. 3C).

Pyrolysis produced partial degradation of lignin side chains, but the aromatic-ring substituents (hydroxy and methoxy groups) remained intact, making possible the identification of products arising from H, G, or S units. Figure 3B shows a series of products characteristic of pyrolysis of phenylpropanoid compounds in wheat straw (i.e., lignin macromolecule and cinnamic acids), including phenol (peak 3), guaiacol (peak 6), and 2,6-dimethoxyphenol (peak 13) and their *para*-methyl (peaks 8 and 16), ethyl (peak 11), vinyl (peaks 9, 12, and 20), and propenyl (peaks 17, 22, and 24) derivatives. These three types of compounds arose from H, G, and S structures, respectively, and the molar composition was estimated from the total abundances of the three types of compounds.

The major products obtained after pyrolysis of the lignin-polysaccharide complex from the lignin medium (Fig. 3D) were nearly identical to those obtained from wheat lignin (Fig. 3B), although three small peaks (peaks 1, 10, and 19) derived from the polysaccharide fraction were also present. Finally, the Py-GC-MS analysis demonstrated the existence of a lignin

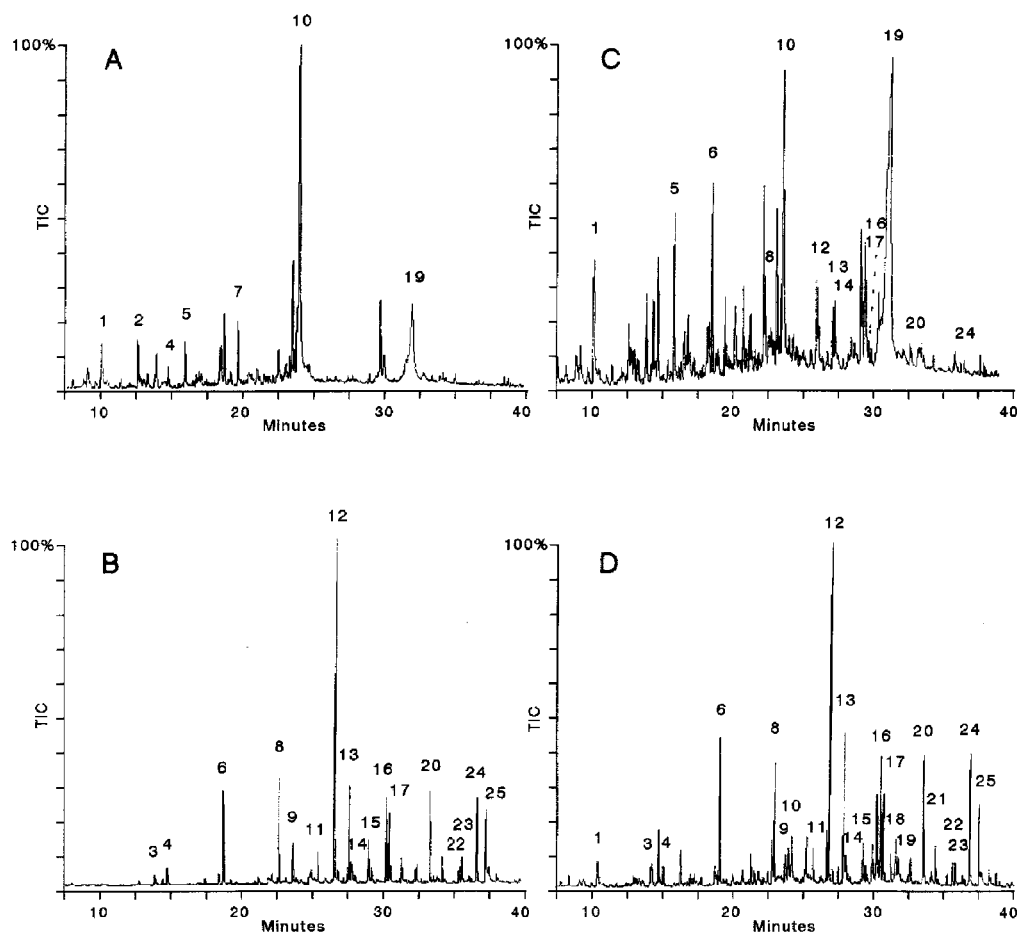


FIG. 3. Chromatograms after Py-GC-MS of the extracellular material isolated from cultures of *P. eryngii* in glucose (A), straw (C), and lignin (D) media and wheat lignin (B). Peaks: 1 = 2,3-dihydro-5-methylfuran-2-one; 2 = 5-methyl-2-furaldehyde; 3 = phenol; 4 = 4-hydroxy-5,6-dihydro-(2H)-piran-2-one; 5 = 2-hydroxy-3-methyl-2-cyclopenten-1-one; 6 = guaiacol; 7 = 2-(propan-2-one)-tetrahydrofuran; 8 = 4-methylguaiacol; 9 = 4-vinyl-phenol; 10 = hydroxymethyl-2-furaldehyde; 11 = 4-ethylguaiacol; 12 = 4-vinyl-guaiacol; 13 = 2,6-dimethoxyphenol; 14 = eugenol; 15 = vanillin; 16 = 2,6-dimethoxy-4-methylphenol; 17 = *trans*-isoeugenol; 18 = acetovanillone; 19 = anhydroglucopyranose; 20 = 2,6-dimethoxy-4-vinylphenol; 21 = *cis*-coniferyl alcohol; 22 = 2,6-dimethoxy-*cis*-4-propenylphenol; 23 = syringaldehyde; 24 = 2,6-dimethoxy-*trans*-4-propenylphenol; 25 = acetosyringone.

fraction in the material obtained from the straw medium (Fig. 3C; peaks 6, 8, 12, 13, 14, 16, 17, 20, and 24).

In agreement with the information provided by CPMAS ^{13}C -NMR, the greatest lignin content (estimated from the total amount of lignin pyrolysis products) corresponded to the complexes formed by *P. eryngii* (in both lignin and straw media). Moreover, Py-GC-MS showed that the composition of the lignin-type fraction from fungal cultures was different from that of the lignin initially added to the medium. In the case of *P. eryngii*, the H/G/S ratio of the aromatic fraction was 4:56:40 in lignin-polysaccharide complexes from lignin medium and 11:73:16 in complexes from straw medium (Fig. 3C and D), in contrast to the ratios found in the controls, which were 8:59:33 and 24:52:24, respectively.

DISCUSSION

The structure of the extracellular polysaccharides produced by the ligninolytic fungi from the genus *Pleurotus* had not been reported previously. This has been due mainly to their low solubility, making them practically insoluble after freeze-drying. The analysis of freeze-dried polysaccharides by FTIR and CPMAS ^{13}C -NMR suggested the presence of β -(1 \rightarrow 3)-glu-

cans, but the presence and nature of branches could not be unequivocally established by these techniques. However, a complete structural characterization was possible after optimizing a dissolution method, which allowed methylation analysis of native and periodate-oxidized polysaccharides. The results obtained showed that the major polysaccharide produced by *Pleurotus* species is a (1 \rightarrow 3)- β -D-glucan, with branches constituted by a glucose residue attached to C-6 every two or three units of the main chain.

In the present study, we also isolated and characterized lignin-polysaccharide complexes formed during wheat lignin degradation by *Pleurotus* species. The above name was applied by analogy with the lignin-polysaccharide complexes extracted from lignocellulosic materials, studied for the first time by Björkman (3). The existence of chemical bonds between the two polymer fractions is suggested by the results of PVP treatment and solvent extraction, which failed to remove the lignin fraction from the polysaccharide polymer. The combination of spectroscopic and degradative techniques, which was found to be appropriate for the characterization of lignin-polysaccharide complexes from wheat straw (7), also provided valuable information on the nature of the complexes formed during lignin degradation by *Pleurotus* species. The spectroscopic

analyses provided information about the nature and general composition of the materials analyzed, while the degradative techniques gave more precise information on the composition and structure of the constituents. Moreover, CPMAS ^{13}C -NMR allowed quantitation of the aromatic moiety, which represents a minor fraction of the complexes obtained from the straw media but amounts to more than 20% of the material recovered from *P. eryngii* cultures in lignin medium.

The structural analyses of the polysaccharide fraction in lignin-carbohydrate complexes were performed by the same methods used for the characterization of the glucans produced in glucose medium. The results showed that the presence of lignin or straw in the culture did not significantly modify the composition or structure of the (1 \rightarrow 6)-branched (1 \rightarrow 3)- β -D-glucan produced by these fungi. This was as expected, since similar extracellular glucans are produced by other fungi in different culture media (1, 34, 35).

Analytical pyrolysis provided the most useful results in the analysis of the aromatic fraction of the lignin-polysaccharide complexes. Only Py-GC-MS had enough sensitivity for the analysis of the aromatic fraction present in the samples from the straw medium. Moreover, the identification of products characteristic of lignin pyrolysis indicated that the aromatic fraction of the complexes consisted of compounds derived from phenylpropanoid compounds (lignin and cinnamic acids) present in wheat straw. When examining their H/G/S composition, it was found that a modification of lignin composition occurred during formation of lignin-polysaccharide complexes. The higher S/G ratio in complexes formed by *P. eryngii* in lignin medium, compared with the control lignin, was estimated by both analytical pyrolysis and CPMAS ^{13}C -NMR spectra (lower relative intensity of the signal at 147 ppm, corresponding to G-units, compared with that at 152 ppm, corresponding to S-units). These differences indicate a differential incorporation of lignin units into the polysaccharide during fungal degradation.

The present study shows that the formation of extracellular polysaccharides constitutes a common characteristic of different species of *Pleurotus*. The (1 \rightarrow 6)-branched (1 \rightarrow 3)- β -D-glucans produced by these fungi are similar to the glucan secreted by *Phanerochaete chrysosporium*, although the latter shows a higher degree of branching (2, 4). The exopolysaccharides are the main constituents of the hyphal sheath, a structure that plays different roles during fungal growth and degradation of different solid substrates (11). By using polysaccharide staining and immunolocalization techniques, it has been demonstrated that the ligninolytic enzymes are fixed on the exopolysaccharide (30). Moreover, it has been suggested that the polysaccharide can participate in lignin degradation by immobilizing degradation products (21). The latter hypothesis is supported by the studies of Kondo et al. (15–18), who demonstrated that the ligninolytic fungi can produce and degrade glycosides of different mono- or polysaccharides and aromatic compounds related to lignin or synthetic lignin. The results obtained here show that during lignin degradation, different aromatic products derived from fungal attack on lignin are incorporated into the extracellular (1 \rightarrow 6)-branched (1 \rightarrow 3)- β -D-glucan synthesized by the ligninolytic fungi of the genus *Pleurotus*.

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